Please amend the application as follows:

In the Claims

9. (Amended) The method of Claim 1 wherein the antigen is [comprises] a T cell epitope.

REMARKS

The remainder of this reply is set forth under appropriate subheadings for the convenience of the Examiner.

Status of Claims

Claims 1-9 and 11-20 are pending in the application. Claim 9 has been amended.

Claim Amendments

Claim 9 has been amended, in accordance with the Examiner's suggestion, to delete the word "comprises," and to recite that the antigen of the claim is a T cell epitope. No new matter has been added by this amendment.

Rejection of Claims 1-9 and 11-20 under U.S.C.§ 103(a)

The Examiner states that, although Applicant's amendment and response (5/22/00) have overcome all previously stated bases of rejection under 35 U.S.C. § 112 and 103, upon reconsideration of the breadth of the claims, prior art is newly cited.

Claim 1 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Wisdom (Ed.) (Peptide Antigens...), hereinafter referred to as "Wisdom", alone or in view of Zegers *et al.* (Immunological Recognition...), hereinafter referred to as "Zegers *et al.*"

Before a consideration of the reference teachings, the Examiner notes that the claims are of sufficient breath to encompass the mere *in vitro* screening of a polypeptide antigen or fragments thereof which comprise T-cell epitopes. The Examiner asserts that there is nothing in the disclosure that defines a vaccine composition as requiring that anything more than any

peptide fragment of an antigen that may or may not represent a T-cell epitope be in the composition.

The Examiner, citing table 1 at page 184 and text at pages 208-215, states that Wisdom teaches how to identify T-cell epitopes of a polypeptide antigen via the screening of synthetic peptide segments of the polypeptide. The Examiner, states that Wisdom teaches the following with regard to the claim steps:

a) that the peptides must be presented to T-cells by antigen presenting cells (APCs) (p. 182);

b) that T-cell lines or clones may be used and that such must be used for epitope mapping with CD8+ T-cells (Table 1 at page 184; pages 212-213.);

c) detection of the T-cell response via T-cell proliferation (tritiated thymidine incorporation into DNA), lysis of target cells (via 51Cr release) or production of cytokines (e.g. IL-2) (pp. 212-213);

d) that the instant claim language is open to include further steps taught by Wisdom by virtue of reciting "comprising"; and that it then would have been obvious to test these *in vivo* since such testing is conventional and would be required by the FDA.

The Examiner states that while chapter 7 of Wisdom does not specifically mention the identified T-cell epitope would be a potential vaccine, one of ordinary skill would have recognized such a potential use of the epitope. The Examiner continues that Wisdom at page 3, teaches that a synthetic peptide vaccine must incorporate both B and T-cell epitopes, and that such epitopes must be identified (page 107, second par.). The Examiner concludes that epitope screening methods taught in Chapter 7 would be a recognized stage in the screening of potential vaccine compositions for their ability to stimulate a T-cell prior to further assessment *in vivo.*

All dependent claims have been rejected over the teachings of Wisdom. The Examiner states that the method of dependent claims 2, 13 and 15 using human T-cells would have been obvious, in order that one might screen for the epitope that would be recognized by the human host that one desired to immunize. The Examiner also asserts that the method of claims 3, 12

and 15 would have been obvious since human T-cells recognize peptides in the context of self MHC. The Examiner states that in the case of Claim 20, it would been obvious to employ autologues APCs since T-cells best recognize foreign peptides in the context of self MHC which is of the same allotype as that of the host from which the T-cells have been obtained.

The Examiner further asserts that the limitations of CD4+ and CD8+ T-cells, as recited in claims 4, 5, 7 and 19 are taught by Wisdom at page 212; that the sources of APCs recited in claim 6 are conventional and hence obvious; and that the response readouts recited in claims 7 and 8 have been noted supra as taught by Wisdom (pp. 212-213).

Zegers *et al.* is optionally relied upon by the Examiner for providing additional motivation for using functional assays when one desires to identify T-cell epitopes to be vaccines. The Examiner states that Zegers *et al.* teach that either peptides or proteolytic fragments (produced by Cathepsin D) may be used in such assays; and that peptide fragments must be presented with the use of MHC on the surface of APCs (p. 106-col. 1).

Applicant's Invention

The claimed invention comprises a method of assessing the ability of a vaccine composition, including almost any forseen antigenic preparations, to stimulate T cell responses. The method comprises the steps of (a) contacting antigen presenting cells in culture with a vaccine composition selected from among a group of vaccine compositions, thereby, if one or more of the antigens or nucleic acid molecules are taken up and processed by the antigen presenting cells, producing one or more processed antigens; (b) contacting the antigen presenting cells with monoclonal T cells under conditions sufficient for the T cells to respond to one or more of the processed antigens; (c) determining whether the T cells respond to one or more of the processed antigens; whereby if the T cells respond to one or more of the processed antigens, then the vaccine composition stimulates a T cell response; (d) repeating steps (a), (b) and (c) with each additional vaccine ~~composition in the group~~, thereby determining whether each vaccine composition stimulates a T cell response; and, if one or more of the vaccine compositions stimulates a T cell response, (d) selecting at least one vaccine composition which stimulates a T cell response for assessment in one or more animals or human subjects. Active compositions are

selected for further testing in animal or human subjects. The sequence of steps in Applicant's claimed method is important.

Further, Applicant's examples show how protein vaccine compositions can be prepared, including formulations with Iscoms which enabled processing the vaccine for $CD8^+$ CTL recognition.

Advantages of Applicant's Invention

Applicant's invention has many advantages. The claimed method is not limited to vaccine compositions including only synthetic peptides. The claimed method enables the screening of complex candidate vaccine preparations, with or without adjuvant material. These complex candidate vaccine preparations include, for example, killed pathogens; a protein or multiple proteins or peptides derived from a pathogen; nucleic acid molecules encoding proteins derived from the pathogen, such as a plasmid DNA vaccine with or without immunostimulatory sequences or cytokine sequences to enhance immunologic activity; a nucleotide molecule administered in a suitable vector; proteins or fragments thereof, which are unique to diseased cells such as cancer cells; and a portion of a pathogen, such as a viral coat or bacterial membrane. All of the above-listed candidate vaccines can be tested with or without adjuvant material.

The invention solves the problem of identifying vaccines without the cost and time delay of conducting animal trials with each test composition. The problem is solved by first assessing the activity of each test composition in an *in vitro* T cell assay and then selecting active test compositions for further trials in animals or human subjects. The activity of the test compositions is not known before testing in the *in vitro* assay. Negative results, showing that the preparation is not antigenic are important. Compositions that are inactive in the *in vitro* assay can be easily and inexpensively identified and discarded before the *in vivo* testing. With Applicant's claimed method, it is not necessary to determine why a vaccine fails. Rather, a subset of vaccines that has been shown by the method to be qualitatively active and quantitatively better can be immediately pursued .

Thus, Applicant's invention provides an effective and economical method of assessing *in vitro* the human immune response to an experimental vaccine construct. For any given disease or

pathogen, the method of the invention enables the rapid evaluation and comparison of a large number of potential vaccine compositions, much greater than the number which can be evaluated by prior art methods, within a short period of time.

Wisdom

At the outset, it should be noted that the teaching in Wisdom is directed to the use of synthetic peptides that correspond to known epitopes on infectious agents. Although a synthetic peptide constituting the antigenic portion of an immunogenic protein may be attached to a suitable carrier molecule to make a synthetic vaccine, one major problem with this method is that the entire antigenic structure of the protein must be known to make an effective vaccine. Few pathogens have such a well-defined antigenic profile. Chapter 7 of Wisdom, "Epitope Mapping Using Synthetic Peptides," including the sections therein cited by the Examiner, is silent on the use of non-synthetic antigens such as would be encompassed by Applicant's claims.

Applicant emphasizes that, although the synthetic peptides taught by Wisdom could be used as a positive control because they may activate a T-cell clone *in vitro*, it is important to note that synthetic peptides have been found not to be useful as vaccines, due in part to their lack of potency *in vivo,* and to the polymorphic nature of human HLA genes, suggesting that many T cell epitopes must be included in vaccines to induce an immune response in most recipients. Thus, that which is made possible by Applicant's claimed method, the ability to rapidly screen complex vaccine preparations which would be likely to have multiple human T cell epitopes, is very different from the teaching in Wisdom.

Applicant submits that the teaching in Wisdom, using only synthetic peptides in high concentration to show activity *in vitro*, is very elemental science. There is no vaccine manufactured using only individual peptides. Wisdom does not consider a wide variety of possible, actual vaccine compositions, for example, killed pathogens; a protein, multiple proteins, or peptides derived from a pathogen; nucleic acid molecules encoding proteins derived from the pathogen, such as a plasmid DNA vaccine with or without immunostimulatory sequences or cytokine sequences to enhance immunologic activity, administered in a suitable

vector; proteins or fragments thereof, which are unique to diseased cells such as cancer cells; and a portion of a pathogen, such as a viral coat or bacterial membrane.

Further, there is not even the slightest suggestion in Wisdom that any elements included in its teachings and cited by the Examiner should be arranged in the sequence of Applicant's claimed method steps, or that the elements are applicable to non-synthetic antigens. Wisdom is not, therefore, a relevant reference under 35 U.S.C. § 103(a).

In the alternative, however, Applicant urges that the teachings of Wisdom do not render Applicant's invention obvious because they are not enabling of what they disclose. The teachings are nothing more than an invitation to experiment. An examination of Wisdom shows that techniques for the exploitation of peptide antigens were just beginning to be investigated, (See Preface, par. 2). For example, Wisdom teaches that "[a]n area of growing importance is the use of peptides to induce the formation of protective antibodies to pathogens," (p. 3, fourth full par., line 1). Referring to difficulties in T cell epitope scanning, at p. 4, third full paragraph, Wisdom comments,

> [T]he examination of the specificity of T-cell receptors is more complicated as this requires soluble peptide and the use of cells to present the peptide to the T-cells. Nevertheless, progress is being made rapidly."

Wisdom teaches that "[e]xperience has shown that T-cell epitopes are difficult to define and contain several categories. For the production of antibodies, a T-helper cell epitope is necessary," (p. 85, par. 4, line 1).

Wisdom focuses on epitope predictions from (known) primary structure of proteins (Chap. 2); solid-phase peptide synthesis (Chap. 3); and traditional and MAP approaches (Chap. 4).

In Chapter 4, Wisdom teaches (p. 107, second full par.) that "An important requirement for synthetic (emphasis added) peptide vaccine development is the identification of the appropriate B- and T-cell epitopes so that they may be attached to the MAP for experimental verification of vaccine efficiency in a well defined animal model for a specific infectious disease."

In Chapter 7 (p. 212) Wisdom describes the synthesis of cleavable peptides for identification of T-cell epitopes, and comments that "the peptides to be tested are required free in solution (i.e. eluted from the pins) so that they can be presented in association with MHC molecules by antigen-presenting cells," (p. 182, third full par.). Wisdom teaches only a sketchy, general method of epitope mapping using synthetic peptides. "An overall strategy for epitope scanning is shown in Figure 1 and the major steps in the production of the synthetic peptides in Table 1," (p. 183, third full par.). Figure 1, Epitope Scanning, outlines a generalized scheme for scanning proteins of known primary structure. The left side of the Figure specifies identification of antibody epitopes by ELISA, and the right side shows cleavable peptides for use in *in vitro* assays using T-cell clones, cell lines or separated cells. Accordingly, Table 1, outlining general experimental steps, relates primarily to the synthesis of peptides. Following the cleaving of cleavable peptides, step 8b, step 9 provides simply "Apply suitable assay procedure and identify epitope." Step 10 provides "Confirm identity of epitope and investigate further." Wisdom is limited to synthesis and testing of synthetic peptides, and includes the caveat, "[a] particular sequence may produce a false negative result in a chosen assay method, if it is a T-cell epitope but produced suboptimally," (p. 209, second full par., last two lines). Further, Wisdom teaches, p. 212, line 1, that "[t]he detection of T-cell epitopes is technically more demanding than identification of antibody epitopes...." Thus, there is no enabling disclosure in Wisdom providing details of epitope scanning techniques. Further, given the comments in Wisdom regarding "false negative results," and other difficulties in detecting T-cell epitopes, it is reasonable to conclude that the teachings of Wisdom do not show a reasonable predictability of success in the field of T-cell epitope scanning.

Applicants respectfully disagree with the Examiner's assertion that " Wisdom teaches how to identify T-cell epitopes of a polypeptide antigen via the screening of synthetic peptide segments of the polypeptide." First, based on the foregoing discussion, it is reasonable to conclude that Wisdom's teaching is simply not enabling of how to carry out the identification. Examination of the specificity of T-cell receptors is more complicated as this requires soluble peptide and the use of cells to present the peptide to the T-cells. Second, Wisdom's scheme is limited to epitopes of a polypeptide antigen of known amino acid sequence. Further , Applicant's

examples show that a protein or inactive virus alone, will not be taken up and presented by APC to activate CD8 + T cells unless incorporated into certain vaccine compositions, for example, including Iscom. Wisdom does not teach how to accomplish this. Wisdom does not teach concerning complex vaccine compositions, with or without adjuvant material, which would allow antigens or nucleic acid molecules to be taken up and processed by antigen presenting cells for T cell recognition.

Further, all of Applicant's claim limitations, in the sequence of steps as listed, are not taught or suggested by Wisdom. For example, there is no particular teaching in Wisdom directing the skilled person to contact antigen presenting cells in culture with a vaccine composition including one or more antigens or one or more nucleic acid molecules encoding one or more antigens, thereby, if one or more of the antigens are taken up by the antigen presenting cells, producing one or more processed antigens; contact the antigen presenting cells with monoclonal T-cells under conditions sufficient for the T cells to respond to the processed antigen; determine whether the vaccine composition is capable of stimulating a T cell response, said determination based upon the T cells responding to the processed antigen; and finally, if a T-cell response has been stimulated by the vaccine composition, assess the vaccine composition in animal or human subjects.

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art, *(In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)). There is no enabling teaching in Wisdom of Applicant's claim limitations. Accordingly, Applicant's claims are not anticipated by or obvious from Wisdom.

In making the obviousness rejection, the Examiner has read the prior art with the benefit of Applicant's disclosure in which there is a clear teaching of the desirability of the method for assessing the ability of a vaccine composition to stimulate a T cell response as set forth in Applicant's claims. In *National Diary Products Corp v. Borden Co. (* 157 USPQ 227, 230 (Ct. App. 1968), the court stated that:

> [t]here is no doubt, . . . that the fact that the solution to a problem is simple, or appears so, when viewed in retrospect, does not mean the solution was obvious when it was made, and that courts must guard against the exercise of hindsight in assessing the obviousness of a given improvement in the art (*Id.* at 231).

Using Applicant's disclosure as a blueprint to reconstruct the claimed invention from isolated pieces of the prior art contravenes the statutory mandate of §103 of judging obviousness

at the point in time when the invention was made. See *Grain Processing Corp. v. American Maize-Prods. Co.*, 840 F.2d 902, 907, U.S.P.Q.2d 1788, 1792 (Fed.Cir. 1988). Applicant respectfully submits that the Examiner's conclusion of obviousness is based upon the considerable benefit of hindsight reasoning, which is improper. The teachings of Wisdom are merely an invitation to explore a promising new field , and do not provide guidance as to the particular form of the claimed invention or how to achieve it. See, for example, *In re O'Farrell*, 853 F.2d 894, 903, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988).

The real potential use of Applicant's claimed method is to examine all complex experimental vaccines, including proteins and plasmid DNA constructs that might be useful in vaccine compositions. The claimed method is new and non-obvious over Wisdom and Zegers *et al.*

Zegers *et al.*

We also respectfully disagree that the teaching in Zegers *et al.*, either alone or in combination with Wisdom, renders Applicant's invention obvious. Zegers *et al.* analyze functionally recognized T helper determinants, but do not remedy the deficiencies of Wisdom. As with the teaching in Wisdom, the teaching in Zegers *et al.* is directed to the use of synthetic immunogen constructs. As stated above, the major problem with the use of synthetic peptides is that the entire antigenic structure of the protein must be known to make an effective vaccine. As with Wisdom, there is not the slightest suggestion in Zegers *et al.* that any elements included in its teachings and cited by the Examiner should be arranged in the sequence of Applicant's claimed method steps, or that the elements are applicable to non-synthetic antigens. Zegers *et al.* therefore, is not a relevant reference under 35 U.S.C. § 103(a).

In addition, Applicant urges that the teachings of Zegers *et al.* do not render Applicant's invention obvious because they are not enabling of what they disclose. Zegers *et al.* teach that "[t]he peptide-MHC complex is recognized by the T cell receptor (TCR) at the T cell. Therefore, a T cell determinant contains at least two domains: one which binds to the groove of the MHC molecule and one for interaction with the TCR." (p. 105, column 1.) Zegers *et al.*, also disclose a construct in which either peptides or proteolytic fragments (produced by Cathepsin D) are used in assays to determine T cell epitopes to be used in vaccines, and in which peptide fragments are

presented with the use of MHC on the surface of APCs (p. 106-col. 1). However, as is true of the teaching in Wisdom, the teaching in Zegers *et al.* is not enabling of how to carry out the determination. Further, all of Applicant's claim limitations, including the claimed sequence of steps, are not taught or suggested by Zegers *et al.*.

Zegers *et al.* teach that "most theoretical prediction methods are merely based on the feasibility of peptide binding in the groove of the MHC molecule, ignoring the interaction with the TCR." (p. 106, par. 4, last sentence.) Zegers *et al.*, (p. 120, last sentence) conclude that the construct "most appropriate on the basis of theoretical choices still needs confirmation of the putative superiority in a functional assay where it is compared to alternative simple constructs." The teachings in Zegers *et al.*, including this conclusion regarding the need for functional assays to confirm the theoretical predictions, do not show a sufficient basis for a reasonable expectation of success in the field of T-cell epitope scanning prior to testing in an animal model. In the present case, the expectation of success is not found in the prior art, but rather in Applicant's disclosure.

Even if one of ordinary skill in the art were to do so, the combination of Wisdom in view of Zegers *et al.* still would not render Applicant's instant invention obvious. Applicant's claimed sequence of steps is not found in either of the references separately, and therefore, would be lacking in any combination of the references. While, based on hindsight reasoning, it may be argued that the need for some of the elements of Applicant's claims is obvious, the prior art references, either considered separately or in combination, do not teach or suggest all the elements of Applicant's claims, as amended.

If an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious, (*In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988). It is, therefore, respectfully requested that the Examiner withdraw the rejection of Applicant's claims under 35 U.S.C. § 103.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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